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A case of cohesinopathy with a novel de-novo *SMC1A* splice site mutation

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List of key features

Developmental delay
Microcephaly
Dysmorphism
Congenital diaphragmatic hernia
Generalized epilepsy
Unilateral camptodactyly of fifth finger

Short clinical summary

The patient's mother reported a twin pregnancy with missed abortion of one of the twins around the sixth week of gestation. Shortly before birth, a prenatal ultrasound revealed a diaphragmatic hernia and microcephaly in the remaining twin. The female patient was born at the 41st week of gestation. Her birth weight was 2720 g (P10) and her body length was 50 cm (P50). Postpartal investigation revealed congenital hip dysplasia. The diaphragmatic hernia was operated upon at the age of 2 days. The body length and weight consistently developed close to the 10th centile, whereas the head circumference was constantly below the third. The developmental milestones were delayed (free walking at 18 months and first words at 2½ years). The first epileptic seizures occurred at the age of 3 months. The girl had generalized tonic–clonic seizures, occasionally occurring in impressive clusters lasting 24–48 h as well as clusters of seizures with secondary generalization. The electroencephalography showed nonspecific monomorphic generalized slow-wave activity with rare spikes. The parents never observed febrile seizures but reported seizures in connection with a sinking body temperature after febrile infections. Genetic analysis revealed a normal female karyotype of 46,XX and normal array comparative genomic hybridization results.

On examination at the age of 11 years, the patient measured 135 cm and weighed 31.5 kg (both P10–P25) with a head circumference of 49.5 cm (P1, −2.5 SD). The dysmorphic features included a round face with arched eyebrows, a short nose, upslanting palpebral fissures, a smoothed philtrum, mild retrognathia, crowded teeth, a flattened midface, clinodactyly of the fifth fingers and camptodactyly of the fifth finger on the right (Fig. 1a–c). There were no flexion deficits of the elbows.

The patient was the second daughter of healthy and nonconsanguineous parents of Swiss origin. The family history was unremarkable, apart from two spontaneous abortions (at approximately the 12th and sixth weeks of gestation) before the patient's healthy elder sister.

Investigations

DNA samples of the patient and both parents were isolated from peripheral blood leucocytes using routine procedures. Molecular genetic analysis of 323 genes involved in epileptogenesis using a targeted next generation sequencing (NGS) approach (epilepsy panel version 2) was performed as recently described (Lemke *et al.*, 2012). Validation of the patient's mutation and analysis of its parental origin was performed by classical Sanger sequencing. We further evaluated the effect of the detected mutation by different in-silico prediction tools (MutationTaster, Human Splicing Finder) (Desmet *et al.*, 2009; Adzhubei *et al.*, 2010; Schwarz *et al.*, 2010).

Results

Within the 323 genes investigated by the NGS epilepsy panel, we revealed 101 variants with a global minor allele frequency of less than 5%. We disregarded the following: (a) variants within the 5′ or 3′ untranslated region, (b) intronic variants more than ±20 bp outside of the common splice sites, (c) synonymous exonic variants more than ±2 bp outside of the common splice sites and (d) variants that were evaluated as being most probably false positives/technical artefacts (e.g. variants present only in forward or reverse reads, those at adjacent positions and those present in <20% of reads).

Among the remaining 12 variants, four were novel to dbSNP 135 (*http://www.ncbi.nlm.nih.gov/projects/SNP/*) (Table 1). Ten of these 12 variants were unlikely to be causative because of two or more of the following reasons: (a) there was only one heterozygous variant within a recessive gene, (b) the gene was not suggestive of the patient's phenotype and (c) the reference sequence presented with the minor allele.

Two variants in *SCN9A* and *SMC1A* were novel to dbSNP 135 and the exome variant server (Seattle, Washington, USA; *evs.gs.washington.edu/EVS/*) and remained with unclear significance.

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Fig. 1

(a) The patient has a round face, arched eyebrows, short nose, upslanting palpebral fissures, smoothed philtrum and crowded teeth; (b) mild retrognathia and a flattened midface and (c) clinodactyly of the fifth fingers and camptodactyly of the fifth finger on the right.

Table 1 List of detected variants

Gene	Mutation	Change of amino acid	Functional class	ID dbSNP 135	Associated disorder	Inheritance	Evaluation
ALDH4A1	c.678+3G>A	NA	Intronic	rs138788183	Hyperprolinaemia type II	AR	AF 0.6% No second mutation
ALG12	c.768+17G>A	NA	Intronic	rs145358718	CDG1G	AR	No second mutation
ALG6	C167+19G>C	NA	Intronic	Novel	CDG1C	AR	No second mutation
ASPM	c.7737T>C	p.Y2494H	NS, coding	rs964201	Microcephaly	AR	Reference has minor allele No second mutation
EIF2B1	c.615G>C	p.D158H	NS, coding	Novel	Leucoencephalopathy with vanishing white matter	AR	No second mutation
EIF2B4	c.704G>A	p.A235T	NS, coding	rs41288829	Leucoencephalopathy with vanishing white matter	AR	AF>3% No second mutation
LAMA2	c.9200–15C>A	NA	Intronic	rs55776770	Merosin-deficient muscular dystrophy	AR	No second mutation
NRXN1	c.2745C>T	p.P469S	NS, coding	rs78540316	Pitt–Hopkins-like syndrome	AR	No second mutation
PAX6	c.129+9G>A	NA	Intronic	rs56139994	Aniridia	AR/(AD)	AF 4.2% No second mutation
RELN	c.5268C>G	p.P1703R	NS, coding	rs2229860	Lissencephaly	AR	AF 1.7% No second mutation
SCN9A	c.1154T>G	p.V385G	NS, coding	Novel	Generalized epilepsy with febrile seizures plus	AD	Inherited paternally
SMC1A	c.1731G>A	p.E577E	S, coding, splice site	Novel	Cornelia de Lange syndrome-2	XD	*De novo*

All 12 detected variants within the targeted 323 genes that remained after the filtering procedure, 10 of which were unlikely to be causative are shown. Of the remaining two variants, only *SCM1A* c.1731G>A appeared to be of de-novo origin and compatible with the girl's phenotype.
AD, autosomal dominant; AF, allele frequency; AR, autosomal recessive; CDG, congenital disorder of glycosylation; NA, not applicable; NS, nonsynonymous; S, synonymous; XD, X-linked dominant.

Finally, the synonymous heterozygous splice variant *SMC1A* c.1731G > A/p.E577E at the last position of exon 10 was proven to be of de-novo origin and predicted to cause a loss of the donor splice site motif. The heterozygous variant *SCN9A* c.1154T > G/p.V385G within exon 9 was predicted to be pathogenic as well but was inherited from the healthy father. Thus, we evaluated the *SCN9A* variant as a presumably rare polymorphism and the de-novo *SMC1A* variant as the most likely causative, leading to the diagnosis of cohesinopathy in our patient. Because of the lack of further consent, additional mRNA studies could not be performed to further support this conclusion.

Discussion

Retrospectively, the girl's symptoms and facial gestalt are compatible with mild Cornelia de Lange syndrome (CdLS). However, we did not consider this diagnosis initially, and thus did not primarily commence with direct genetic testing of *SMC1A*. Unfortunately, this is a frequent situation in clinical genetics, as symptoms and features of a patient only rarely allow a concise and correct diagnosis and often require considerable syndromatological knowledge. Hence, targeted NGS can release clinicians from the prerequisite of a precise clinical diagnosis before genetic testing.

Most known *SMC1A* patients have been detected because of their CdLS/CdLS-like phenotypes (Deardorff *et al.*, 2007). However, several patients lacking classical or suggestive phenotypic features (Deardorff *et al.*, 2007) prove that the spectrum of *SMC1A* mutations might go beyond the known CdLS-like phenotype. This is supported by the case of a recently described nonsyndromic, autistic patient carrying a *SMC3* mutation (Sanders *et al.*, 2012). Thus, a direct clinical diagnosis can be challenging and the broader term 'cohesinopathy' might be more adequate for this phenotypic spectrum than is CdLS.

The mutation c.1731G > A/p.E577E in our patient expands the mutational spectrum of *SMC1A* to splice site mutations and also represents the first exonic synonymous splice site mutation observed in any human cohesinopathy.

The detected variant *SCN9A* p.V385G remains of unknown significance. Because of paternal inheritance, it is unlikely to be causative of the girl's phenotype but might still modify her generalized epilepsy (Singh *et al.*, 2009).

Congenital diaphragmatic hernias have been described occasionally in patients with 'classic' CdLS because of *NIPBL* mutations (Fryns, 1987). However, Deardorff *et al.* (2007) reported no diaphragmatic hernias nor other major structural abnormalities in *SMC1A* (and *SMC3*) patients. Our observation reveals that diaphragmatic hernias are also part of the milder (non-*NIPBL*) cohesinopathies.

Finally, we demonstrate how NGS panel diagnostics significantly facilitate mutation detection. Screening of larger cohorts with less specific phenotypes might help reveal the true phenotypic spectrum of many conditions, not only of human cohesinopathies or epilepsies. As shown in several cases, it will dramatically expand our understanding of the genotype–phenotype correlations of known (and yet unknown) disease-causing genes (Kousi *et al.*, 2012; Lemke *et al.*, 2012).

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Conflicts of interest

There are no conflicts of interest.

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